

T7 primer
TTAATACGACTCACTATAGGG AGACCGG AAG CTT AAG GTG CAC GGC CCA CGT GGA TCG ATC GCG CGC AGA TCT TCG GAA
Hind 3 Bgl II

-28
Met Asp Ser Lys Val Thr Ile Ile Cys Ile Arg Phe Leu Phe Trp Phe Leu Leu Leu Cys Met Leu Ile Gly Lys Ser His Thr
GCC ACC ATG GAT AGC AAA GTC ACA ATC ATA TGC ATC AGA TTT CTC TTT TGG TTT CTT TTG CTC TGC ATG CTT ATT GGG AAG TCA CAT ACT
+1 NcoI 10 20 30
Glu Asp Asp Ile Ile Ile Ala Thr Lys Asn Gly Lys Val Arg Gly Met Asn Leu Thr Val Phe Gly Gly Thr Val Thr Ala Phe Leu Gly
GAA GAT GAC ATC ATA ATT GCA ACA AAG AAT GGA AAA GTC AGA GGG ATG AAC TTG ACA GTT TTT GGT GGC ACG GTA ACA GCC TTT CTT GGA 90
40 50 60
Ile Pro Tyr Ala Gln Pro Pro Leu Gly Arg Leu Arg Phe Lys Lys Pro Gln Ser Leu Thr Lys Trp Ser Asp Ile Trp Asn Ala Thr Lys
ATT CCC TAT GCA CAG CCA CCT CTT GGT AGA CTT CGA TTC AAA AAG CCA CAG TCT CTG ACC AAG TGG TCT GAT ATT TGG AAT GCC ACA AAA 180
Eco RI Acc I Taq I 80 90
Tyr Ala Asn Ser Cys Cys Gln Asn Ile Asp Gln Ser Phe Pro Gly Phe His Gly Ser Glu Met Trp Asn Pro Asn Thr Asp Leu Ser Glu
TAT GCA AAT TCT TGC TGT CAG AAC ATA GAT CAA AGT TTT CCA GGC TTC CAT GGA TCA GAG ATG TGG AAC CCA AAC ACT GAC CTC AGT GAA 270
100 110 120
Asp Cys Leu Tyr Leu Asn Val Trp Ile Pro Ala Pro Lys Pro Lys Asn Ala Thr Val Leu Ile Trp Ile Tyr Gly Gly Phe Gln Thr
GAC TGT TTA TAT CTA AAT GTA TGG ATT CCA GCA CCT AAA CCA AAA AAT GCC ACT GTA TTG ATA TGG ATT TAT GGT GGT GGT TTT CAA ACT 360
130 140 150
Gly Thr Ser Ser Leu His Val Tyr Asp Gly Lys Phe Leu Ala Arg Val Glu Arg Val Ile Val Val Ser Met Asn Tyr Arg Val Gly Ala
GGA ACA TCA TCT TTA CAT GTT TAT GAT GGC AAG TTT CTG GCT CCG GTT GAA AGA GTT ATT GTA GTG TCA ATG AAC TAT AGG GTG GGT GCC 450
160 170 180
Leu Gly Phe Leu Ala Leu Pro Gly Asn Pro Glu Ala Pro Gly Asn Met Gly Leu Phe Asp Gln Gln Leu Ala Leu Gln Trp Val Gln Lys
CTA GGA TTC TTA GCT TTG CCA GGA AAT CCT GAG GCT CCA GGG AAC ATG GGT TTA TTT GAT CAA CAG TTG GCT CTT CAG TGG GTT CAA AAA 540
190 200 210
Asn Ile Ala Ala Phe Gly Gly Asn Pro Lys Ser Val Thr Leu Phe Gly Glu Ser Ala Gly Ala Ala Ser Val Ser Leu His Leu Leu Ser
AAT ATA GCA GCC TTT GGT GGA AAT CCT AAA AGT GTA ACT CTC TTT GGA GAA AGT GCA GGA GCA GCT TCA GTT AGC CTG CAT TTG CTT TCT 630
220 230 240
Pro Gly Ser His Ser Leu Phe Thr Arg Ala Ile Leu Gln Ser Gly Ser Phe Asn Ala Pro Trp Trp Ala Val Thr Ser Leu Tyr Glu Ala Arg
CCT GGA AGC CAT TCA TTG TTC ACC AGA GCC ATT CTG CAA AGT GGT TCC TTT AAT GCT CCT TGG GCG GTA ACA TCT CTT TAT GAA GCT AGG 720
250 260 270
Asn Arg Thr Leu Asn Leu Ala Lys Leu Thr Gly Cys Ser Arg Glu Asn Glu Thr Glu Ile Ile Lys Cys Leu Arg Asn Lys Asp Pro Gln
AAC AGA ACG TTG AAC TTA GCT AAA TTG ACT GGT TGC TCT AGA GAG AAT GAG ACT GAA ATA ATC AAG TGT CTT AGA AAT AAA GAT CCC CAA 810
280 290 300
Glu Ile Leu Leu Asn Glu Ala Phe Val Val Tyr Gly Thr Pro Leu Ser Val Asn Phe Gly Pro Thr Val Asp Gly Asp Phe Leu Thr
GAA ATT CTT CTG AAT GAA GCA TTT GTT GTC CCC TAT GGG ACT CCT TTG TCA GTA AAC TTT GGT CCG ACC GTG GAT GGT GAT TTT CTC ACT 900
310 320 330
Asp Met Pro Asp Ile Leu Leu Glu Leu Gly Gln Phe Lys Lys Thr Gln Ile Leu Val Gly Val Asn Lys Asp Glu Gly Thr Thr Phe Leu
GAC ATG CCA GAC ATA TTA CTT GAA CTT GGA CAA TTT AAA AAA ACC CAG ATT TTG GTG GGT GTT AAT AAA GAT GAA GGG ACA TGG TTT TTA 990
340 350 360
Val Tyr Gly Ala Pro Gly Phe Ser Lys Asp Asn Asn Ser Ile Ile Thr Arg Lys Glu Phe Gln Glu Gly Leu Lys Ile Phe Phe Pro Gly
GTC TAT GGT GCT CCT GGC TTC AGC AAA GAT AAC AAT AGT ATC ATA ACT AGA AAA GAA TTT CAG GAA GGT TTA AAA ATA TTT TTT CCA GGA 1080
370 380 390
Val Ser Glu Phe Gly Lys Glu Ser Ile Leu Phe His Tyr Thr Asp Trp Val Asp Asp Gln Arg Pro Glu Asn Tyr Arg Glu Ala Leu Gly
GTG AGT GAG TTT GGA AAG GAA TCC ATC CTT TTT CAT TAC ACA GAC TGG GTA GAT GAT CAG AGA CCT GAA AAC TAC CGT GAG GCC TTG GGT 1170
400 410 420
Asp Val Val Gly Asp Tyr Asn Phe Ile Cys Pro Ala Leu Glu Phe Thr Lys Lys Phe Ser Glu Trp Gly Asn Asn Ala Phe Phe Tyr Tyr
GAT GTT GTT GGG GAT TAT AAT TTC ATA TGC CCT GCC TTG GAG TTC ACC AAG AAG TTC TCA GAA TGG GGA AAT AAT GCC TTT TTC TAC TAT 1260
430 440 450
Phe Glu His Arg Ser Ser Lys Leu Pro Trp Pro Glu Trp Met Gly Val Met His Gly Tyr Glu Ile Glu Phe Val Phe Gly Leu Pro Leu
TTT GAA CAC CCA TCC TCC AAA CTT CCG TGG CCA GAA TGG ATG GGA GTG ATG CAT GGC TAT GAA ATT GAA TTT GTC TTT GGT TTA CCT CTG 1350
460 470 480
Glu Arg Arg Asp Asn Tyr Thr Lys Ala Glu Glu Ile Leu Ser Arg Ser Ile Val Lys Arg Trp Ala Asn Phe Ala Lys Tyr Gly Asn Pro
GAA AGA AGA GAT AAT TAC ACA AAA GCC GAG GAA ATT TTG AGT AGA TCC ATA GTG AAA CCG TGG GCA AAT TTT GCA AAA TAT GGG AAT CCA 1440
490 500 510
Asn Glu Thr Gln Asn Asn Ser Thr Ser Trp Pro Val Phe Lys Ser Thr Glu Gln Lys Tyr Leu Thr Leu Asn Thr Glu Ser Thr Arg Ile
AAT GAG ACT CAG AAC AAT AGC ACA AGC TGG CCT GTC TTC AAA AGC ACT GAA CAA AAA TAT CTA ACC TTG AAT ACA GAG TCA ACA AGA ATA 1530
520 530 540
Met Thr Lys Leu Arg Ala Gln Gln Cys Arg Phe Trp Thr Ser Phe Phe Pro Lys Val Leu Glu Met Thr Gly Asn Ile Asp Glu Ala Glu
ATG ACG AAA CTA CGT GCT CAA CAA TGT CGA TTC TGG ACA TCA TTT TTT CCA AAA GTC TTG GAA ATG ACA GGA AAT ATT GAT GAA GCA GAA 1620
550 560 570
Trp Glu Trp Lys Ala Gly Phe His Arg Trp Asn Asn Tyr Met Met Asp Trp Lys Asn Gln Phe Asn Asp Tyr Thr Ser Lys Lys Glu Ser
TGG GAG TGG AAA GCA GGA TTC CAT CGC TGG AAC AAT TAC ATG ATG GAC TGG AAA AAT CAA TTT AAC GAT TAC ACT AGC AAG AAA GAA AGT 1710
574
Cys Val Gly Leu ***
TGT GTG GGT CTC TAA TTA ATA GAT CTG TCA TGA TGA TCA TTG CAA TTG GAT CCA TAT ATA GGG CCC TATT CTATAGTGTACCTAAAT
Ase I Bgl II Bcl I Bam HI, Eco01091, Apa I Sp6 primer

Figure 1. Nucleic acid sequence and deduced amino acid sequence of the human butyrylcholinesterase variant A328W. Position 328 is enclosed in boxes. The sequence has been modified in two additional ways: 1) the naturally occurring Bam HI site at position 225-226 has been removed without changing the amino acids Gly-Ser. 2) The sequence surrounding the translation initiation site, ATG at position -28, has been optimized to fit the Kozak rules (Kozak M. 1991, Journal of Biological Chemistry 266: 19867-19870) for the purpose of obtaining maximal levels of expression.

FIGURE 1

Inventors: Lockridge and Watkins
P-IX 4143

EDDIIIATKNGKVRGMNLTVFGGTVTAFLGIPYAQPPLGRLRFKKPQSLTK
WSDIWNATKYANSCCONIDOSEFPGFHGSEMWNPNNTDLSCLYLNWVWIPAP
KPKNATVLIWIIYGGGFOTGTSSLHVYDYGKFLARVERVIVVSMNYRVGALGF
LALPGNPEAPGNMGLFDQQLALQWVQKNIAAFGGNPKSVTLFGESAGAASV
SLHLLSPGSHSLFTRAILQSGSFNAPWAVTSLYEARNRTLNLAKLTGCSRE
NETEIIKCLRNKDPQEILLNEAFVVPYGTPLSVNFGPTVDGDFLTDMPDIL
LELGQFKKTQILVGVNKDEGTAEFLVYGAPGFSKDNNSSIITRKEFQEGLKIF
FPGVSEFGKESILFHYTDWVDDQRPENYREALGDVVGDDYNFICPALEFTKK
FSEWGNNAFFYYFEHRSSKLPWPPEWMGVMHGYEIEFVFGGLPLERRDNYTKA
EEILSRSIVKRWANFAKYGNPNETQNNSTSWPVFKSTEQKYLTNTTESTRI
MTKLRAQQCRFWTSFFPKVLEMTGNIDEAEWEWKAGFHRWNNYMMDWKNQF
NDYTSKKESCVGL

FIGURE 2

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1  tactgaatgt cagtgcagtc caatttacag gctggagcag cagctgcata ctgcatttcc
61 ccgaagtatt acatgatttt cactccttgc aaactttacc atctttgttg cagagaatcg
121 gaaatcaata tgcatagcaa agtcacaatc atatgcatca gatttctctt ttggtttctt
181 ttgctctgca tgcttattgg gaagtcacat actgaagatg acatcataat tgcaacaaag
241 aatggaaaag tcagagggat gaacttgaca gtttttgggt gcacggtaac agcctttctt
301 ggaattccct atgcacagcc acctcttggg agacttcgal tcaaaaagcc acagtctctg
361 accaagtggg ctgatatttg gaatgccaca aaatatgcaa attcttgctg tcagaacata
421 gatcaaagtt ttccaggctt ccatggatca gagatgtgga acccaaacac tgacctcagt
481 gaagactggt tatatctaaa tgtatggatt ccagcaccta aacccaaaaa tgccactgta
541 ttgatatgga tttatgggtg tggttttcaa actggaacat catctttaca tgtttatgat
601 ggcaagtttc tggctcgggt tgaaagagtt attgtagtgt caatgaacta taggggtgggt
661 gccctaggat tcttagcttt gccaggaaat cctgaggctc cagggaacat ggggtttattt
721 gatcaacagt tggctcttca gtgggttcaa aaaaatatag cagcctttgg tggaaatcct
781 aaaagtgtaa ctctctttgg agaaagtgca ggagcagctt cagttagcct gcatttgctt
841 tctcctggaa gccattcatt gtaccacaga gccattctgc aaagtggatc ctttaatgct
901 ccttggggcg taacatctct ttatgaagct aggaacagaa cgttgaactt agctaaattg
961 actggttgct ctagagagaa tgagactgaa ataatcaagt gtcttagaaa taaagatccc
1021 caagaaattc ttctgaatga agcatttggt gtccctatg ggactccttt gtcagtaaac
1081 tttgggtccga ccgtggatgg tgattttctc actgacatgc cagacatatt acttgaactt
1141 ggacaattta aaaaaacca gattttgggt ggtgttaata aagatgaagg gacagctttt
1201 ttagtctatg gtgctcctgg cttcagcaaa gataacaata gtatcataac tagaaaagaa
1261 tttcaggaag gtttaaaaat attttttcca ggagtgagtg agtttggaag ggaatccatc
1321 ctttttcatt acacagactg ggtagatgat cagagacctg aaaactaccg tgaggccttg
1381 ggtgatgttg ttggggatta taatttcata tgccctgcct tggagttcac caagaagttc
1441 tcagaatggg gaaataatgc ctttttctac tattttgaac accgatcctc caaacttccg
1501 tggccagaat ggatgggagt gatgcatggc tatgaaattg aatttgtctt tggtttacct
1561 ctggaaagaa gagataatta caaaaagcc gaggaatttt tgagtagatc catagtgaag
1621 cgggtgggcaa attttgcaaa atatgggaat ccaaatgaga ctcagaacaa tagcacaagc
1681 tggcctgtct tcaaaagcac tgaacaaaaa tatctaacct tgaatacaga glcaacaaga
1741 ataatgacga aactacgtgc tcaacaatgt cgattctgga catcattttt tccaaaagtc
1801 ttggaaatga caggaaatat tgatgaagca gaatgggagt ggaaagcagg attccatcgc
1861 tggaacaatt acatgatgga ctggaaaaat caatttaacg attacactag caagaaagaa
1921 agttgtgtgg gtctctaatt aatagattta ccctttatag aacatatttt cctttagatc
1981 aaggcaaaaa tatcaggagc ttttttacac acctactaaa aaagttatta tgtagctgaa
2041 acaaaaatgc cagaaggata atattgatgc ctcacatctt taacttagta ttttacctag
2101 catttcaaaa ccaaatggc tagaacatgt ttaattaaat ttcacaatat aaagttctac
2161 agttaattat gtgcatatta aaacaatggc ctggttcaat ttctttcttt ccttaataaa
2221 ttttaagttt ttcccccaa aattatcagt gctctgctt tagtcacgtg tattttcatt
2281 accactcgta aaaaggtatc ttttttaaat gaattaaata ttgaaacact gtacaccata
2341 gtttacaata ttatgtttcc taattaaaat aagaattgaa tgtcaatatg agatattaaa
2401 ataagcacag aaaatc

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Figure 3. Nucleic acid sequence of human butyrylcholinesterase (SEQ ID NO: 16)
Genbank accession number M16541.

FIGURE 3

					1	10	20	30
human wild-type BChE					EDDIIIATKN	GKVRGMNLTV	FGGTVTAFLG	
human A variant BChE					-----	-----	-----	-----
human J variant BChE					-----	-----	-----	-----
human K variant BChE					-----	-----	-----	-----
rat BChE					EEDVIITTKT	GRVRGLSMPI	LGGTVTAFLG	
cat BChE					EEDIIITTKN	GKVRGMNLPV	LDGTVTAFLG	
horse BChE					EEDIIITTKN	GKVRGMNLPV	LGGTVTAFLG	
		40	50	60	70	80	90	100
human wt	IPYAQPPLGR	LRFKKPQSLT	KWSDIWNATK	YANSCCQNID	QSFPGFHGSE	MWNPNTDLSE	DCLYLNWVWP	
human A	-----	-----	-----	-----G	-----	-----	-----	
human J	-----	-----	-----	-----	-----	-----	-----	
human K	-----	-----	-----	-----	-----	-----	-----	
rat	IPYAQPPLGS	LRFKKPQPLN	KWPDVYNATK	YANSCYQNID	QAFPGFQGSE	MWNPNTNLSE	DCLYLNWVWP	
cat	IPYAQPPLGR	LRFKKPQFLT	KWSDIWNATK	YANSCYQNAD	QSFPGFPGSE	MWNPNTDLSE	DCLYLNWVWP	
horse	IPYAQPPLGR	LRFKKPQSLT	KWSNIWNATK	YANSCYQNTD	QSFPGFGLSE	MWNPNTLSE	DCLYLNWVWP	
		110	120	130	140	150	160	170
human wt	APKPKNATVL	IWIYGGGFQT	GTSSLHVDYD	KFLARVERVI	VVSMNYRVGA	LGFLALPGNP	EAPGNMGLFD	
human A	-----	-----	-----	-----	-----	-----	-----	
human J	-----	-----	-----	-----	-----	-----	-----	
human K	-----	-----	-----	-----	-----	-----	-----	
rat	VPKPKNATVM	VWVYGGGFQT	GTSSLPVYD	KFLTRVERVI	VVSMNYRVGA	LGFLAFPGNS	EAPGNMGLFD	
cat	TPKPKNATVM	IWIYGGGFQT	GTSSLPVYD	KFLARVERVI	VVSMNYRVGA	LGFLALPGNP	EVPGNMGLFD	
horse	APKPKNATVM	IWIYGGGFQT	GTSSLPVYD	KFLARVERVI	VVSMNYRVGA	LGFLALSEN	EAPGNMGLFD	
		180	190	200	210	220	230	240
human wt	QQLALQWVQK	NIAAFGGNPK	SVTLFGESAG	AASVSLHLLS	PGSHSLFTRA	ILQSGSFNAP	WAVTSLYEAR	
human A	-----	-----	-----	-----	-----	-----	-----	
human J	-----	-----	-----	-----	-----	-----	-----	
human K	-----	-----	-----	-----	-----	-----	-----	
rat	QQLALQWVQK	NIAAFGGNPK	SVTLFGESAG	AASVSLHLLC	PQSYPLFTRA	ILES GSSNAP	WAVKHPEEAR	
cat	QQLALQWVQK	NIAAFGGNPK	SVTLFGESAG	AGSVSLHLLS	PRSQPLFTRA	ILQSGSSNAP	WAVMSLDEAK	
horse	QQLALQWVQK	NIAAFGGNPR	SVTLFGESAG	AASVSLHLLS	PRSQPLFTRA	ILQSGSSNAP	WAVTSLYEAR	
		250	260	270	280	290	300	310
human wt	NRTLNLAKLT	GCSRENETEI	IKCLRNDKPQ	EILLNEAFV	PYGTPLSVNF	GPTVDGDFLT	DMPDILLELG	
human A	-----	-----	-----	-----	-----	-----	-----	
human J	-----	-----	-----	-----	-----	-----	-----	
human K	-----	-----	-----	-----	-----	-----	-----	
rat	NRTLTLAKFI	GCSKENEKEI	ITCLRSKDPQ	EILLNEKLVL	PSDSIRSINF	GPTVDGDFLT	DMPHTLLQLG	
cat	NRTLTLAKFI	GCSKENDTEI	IKCLRNDKPQ	EILLNELLV	PSDTLLSVNF	GPVVDGDFLT	DMPDTLLQLG	
horse	NRTLTLAKRM	GCSRDNETEM	IKCLRNDKPQ	EILLNEVFV	PYDTLLSVNF	GPTVDGDFLT	DMPDTLLQLG	
		320	330	340	350	360	370	380
human wt	QFKKTQILVG	VNKDEGTAFL	VYGAPGFSKD	NNSIITRKEF	QEGLKIFFPG	VSEFGKESIL	FHYTDWVDDQ	
human A	-----	-----	-----	-----	-----	-----	-----	
human J	-----	-----	-----	-----	-----	-----	-----	
human K	-----	-----	-----	-----	-----	-----	-----	
rat	KVKTAQILVG	VNKDEGTAFL	VYGAPGFSKD	NDSLITRREF	QEGLNMYFPG	VSSLGKEAIL	FYYVDWLGDQ	
cat	QFKKTQILVG	VNKDEGTAFL	VYGAPGFSKD	NDSIITRKEF	QEGLKIYFPG	VSEFGREAIL	FYYVDLLDDQ	
horse	QFKRTQILVG	VNKDEGTAFL	VYGAPGFSKD	NNSIITRKEF	QEGLKIFFPR	VSEFGRESIL	FHYMDWLDDQ	

FIGURE 4 (PAGE 1 OF 2)

Inventors: Lockridge and Watkins
Docket No.: P-IX 4143

	390	400	410	420	430	440	450
human wt	RPENYREALG	DVVGDDYNFIC	PALEFTKKFS	EWGNNAFFYY	FEHRSSKLPW	PEWMGVMHGY	EIEFVFGGLPL
human A	-----	-----	-----	-----	-----	-----	-----
human J	-----	-----	-----	-----	-----	-----	-----
human K	-----	-----	-----	-----	-----	-----	-----
rat	TPEVYREAFD	DIIGDYNIIIC	PALEFTKKFA	ELEINNAFFYY	FEHRSSKLPW	PEWMGVMHGY	EIEFVFGGLPL
cat	RAEKYREALD	DVLGDYNIIC	PALEFTTKFS	ELGNNAFFYY	FEHRSSQLPW	PEWMGVMHGY	EIEFVFGGLPL
horse	RAENYREALD	DVVGDDYNIIC	PALEFTRKFS	ELGNDAFFYY	FEHRSTKLPW	PEWMGVMHGY	EIEFVFGGLPL
	460	470	480	490	500	510	520
human wt	ERRDNYTKAE	EILSRIVKR	WANFAKYGNP	NETQNNSTSW	PVFKSTEQKY	LTNLNTESTRI	MTKLRAQQCR
human A	-----	-----	-----	-----	-----	-----	-----
human J	-----	-----	-----	-----	-----V-----	-----	-----
human K	-----	-----	-----	-----	-----	-----	-----
rat	ERRVNYTRAE	EIFSRSIMKT	WANFAKYGHP	NGTQGNSTVW	PVFTSTEQKY	LTNLNTEKSKI	NSKLRAQQCQ
cat	ERRVNYTRAE	EILSRSIMNY	WANFAKYGNP	NGTQNNSTRW	PAFRSTDQKY	LTNLNABSPKV	YTKLRAQQCR
horse	ERRVNYTRAE	EILSRSIMKR	WANFAKYGNP	NGTQNNSTRW	PVFKSTEQKY	LTNLNTESPKV	YTKLRAQQCR
	530	540	550	560	570	574	
human wt	FWTSFFPKVL	EMTGNIDEAE	WEWKAGFHRW	NNYMMDWKNQ	FNDYTSKKES	CVGL	
human A	-----	-----	-----	-----	-----	----	
human J	-----	-----	-----	-----	-----	----	
human K	-----	-----T-----	-----	-----	-----	----	
rat	FWRLFFPKVL	EITGDIDERE	QEWKAGFHRW	SNYMMDWKNQ	FNDYTSKKET	CTDL	
cat	FWTLFFPKVL	EMTGNIDEAE	REWRAGFYRW	NNYMMDWKNQ	FNDYTSKKES	CAGL	
horse	FWTLFFPKVL	ELTGNIDEAE	REWKAGFHRW	NNYMMDWKNQ	FNDYTSKKES	CSDF	

FIGURE 4 (PAGE 2 OF 2)



FIGURE 5

Inventors: Lockridge and Watkins
Docket No.: P-IX 4143

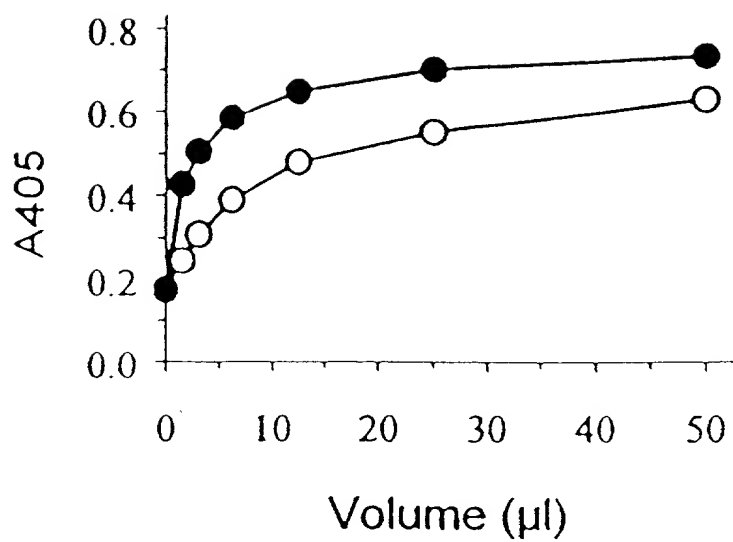


FIGURE 6

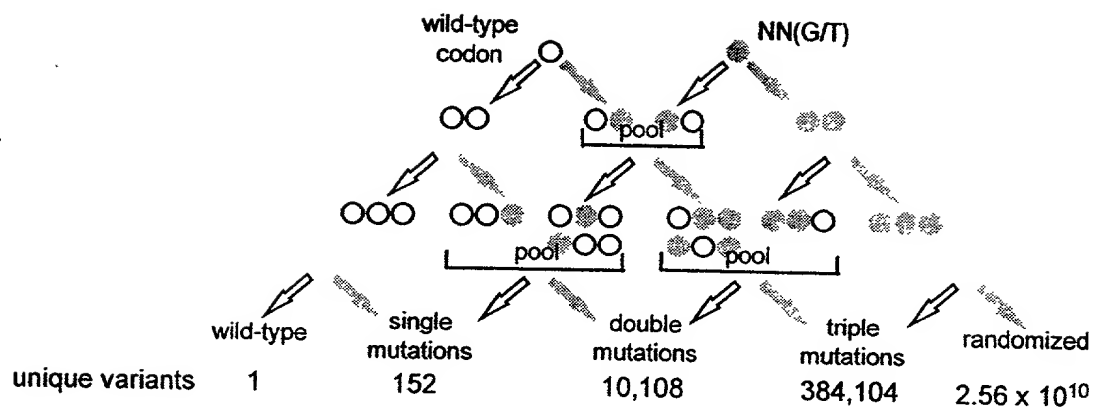


FIGURE 7